COPPER: ITS OCCURRENCE AND RÔLE IN INSECTS AND OTHER ANIMALS[1]

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I. GENERAL

The present paper arose from certain experiments on the respiration of insects, particularly on the gases of the blood, and its rôle as a respiratory factor.

As at present understood, respiration in insects proceeds by the tracheal method: Atmospheric air is led directly to the cells by the tracheae, while the blood acts primarily in the transportation of food and metabolic products. This is modified in aquatic stages, in some of which the oxygen in solution in the water is absorbed by means of tracheal gills. In certain *secondarily* aquatic insects,—that is, insects originally aquatic which became terrestrial in habit, but which in some stages again have sought the water,—there are found structures which fundamentally are of the gill type. Here a thin membrane separates the blood from the water; the blood takes up oxygen through this membrane and distributes it directly to the tissues, or indirectly by yielding it to the gaseous supply in the tracheae. Such structures are the gill filaments and gill pouches of Trichoptera larvae and aquatic caterpillars, the caudal gill pouches of Chironomid larvae, Culicid larvae, Simulium larvae, etc.

Considered from both the physiological and morphological standpoint, these structures are meaningless unless a respiratory protein is postulated in the insect blood to fix the transfusing oxygen. Without such a protein, there could exist only an oxygen balance between the fluids divided by the animal membrane,—between the oxygen in solution in the blood serum and that in solution in the water. But since the available dissolved oxygen decreases with the rising temperature of the water, and the temperature of the insect rises with that of its environment, the oxygen supply of the water becomes impoverished, and with it the amount in solution in the blood,—that is, if the blood lacked a respiratory protein.

[1] (Contribution from the Zoological Laboratory of the University of Idaho, Moscow, Idaho.)

Furthermore, many insects are found in places where the available oxygen is nearly entirely used up in the decomposition and oxidation of organic waste. Indeed, a number of species are known which live, grow, and transform under anaerobic conditions (Juday 1909, Muttkowski, 1918).

Now, a respiratory protein is known for a few insect species, among them some of the anaerobes just referred to, especially the so-called "blood-worms" or Chironomid larvae. This pigment has been identified as hemoglobin, dissolved in the blood plasma, and not included in the corpuscles. As far as known to the writer, it is restricted among insects to the "blood-worm" type of Chironomid larvae. The important point in connection with the hemoglobin of these larvae is this: It is confined to a few species, but not all of these species live under anaerobic conditions; nor do all anaerobic Chironomids contain hemoglobin. One is forced to the conclusion that among these latter the hemoglobin is replaced by some other respiratory pigment. Hemocyanin has been suggested by the writer (1920), altho it had not been demonstrated for a single insect species.

It is known from the study of vertebrate blood that hemoglobin forms oxyhemoglobin with oxygen and carbohemoglobin with carbon dioxide. From its identity with vertebrate hemoglobin it can be supposed that the activity of the hemoglobin as found in the Chironomid "blood-worms" is similarly two-fold,—that it transports both oxygen and carbon dioxide. For the rest of insects it was shown by the writer that both oxygen and carbon dioxide are present in the blood (account published elsewhere). Hence it is logical to assume that, similar to Chironomid "blood-worms," there is a definite carrier which fixes both oxygen and carbon dioxide.

The following recounts a series of experiments undertaken in an attempt to prove or disprove the foregoing assumption. The experiments were performed during the spring, summer and fall of 1920, altho some earlier observations made in the course of the past ten years are included.

II. Respiratory Proteins in Insects; the Copper Nucleus

In its development the problem presented several distinct phases: (1) Aside from the few insects possessing hemoglobin, is a respiratory protein available at least in those aquatic insects provided with blood gills? (2) If such a protein can be demonstrated, what is its nature? (3) If available, is it confined to aquatic stages or is its distribution universal among insects?

The presence of hemoglobin in Chironomid larvae (blood-worm type) is easily verified. For the blood responds to the various oxidation (Guiac, O-tolidin, and Benzidine reactions) and crystallization tests (Hemin) that have been elaborated for the recognition and study of hemoglobin in vertebrate blood. Except for the hemin test, none of these is conclusive

as far as general differentiation between Invertebrate and Vertebrate blood is concerned. The hemin test alone indicates positively the presence of hemoglobin *as such* in Chironomid "blood-worms," or any other animal. Yet in a number of tests made for hemoglobin in the colorless blood of species like Anax, Aeshna, Dytiscus, and others, isolated crystals other than Sodium chloride were found which resembled the familiar prisms of hemin.

The oxidation tests are conclusive only in so far as they reveal the presence of blood, specifically the respiratory protein. They do not indicate the identity of this pigment. For it is noteworthy that the blood of crayfish as well as that of all insects reacts with Guaiac, and Benzidine, and produces color changes identical with those produced by vertebrate blood (See table I). Note that these oxidations are produced by blood which shows little or no trace of hemoglobin. Furthermore, as with vertebrate blood, boiling the test substance does not stop the reaction.

TABLE I. REACTIONS OF INSECT BLOOD TO HEMOGLOBIN TESTS

Name of Species	Guiac Test (Oxidation)		Benzidine Test (Oxidation)		Hemin Test-Crystals (Nippe's Reagent)	
	Number	Result	Number	Result	Number	Result
Aeshna larva.......	8	pos.	3	pos.	5	4 neg., 1 trace
Anax, yg. larvae....	2	"	1	"		
Enallagma larvae...	2	"	1	"		
Mayfly nymphs....	1	"				
Belostoma.........	3	"	1	"	7	5 neg., 2 trace
Chironomus larvae	6	"	3	"	12	12 pos.
Dytiscus larvae....	2	"			6	5 neg., 1 trace
Dytiscus adults....	2	"	1	"	7	5 neg., 2 trace
Controls						
Cambarus blood...	6	"	3	"	5	5 neg.
Blank, with $FeCl_2$..	5	"	3	"		
Blank, with $CuSO_4$	5	"	3	"		

In hemoglobin the iron is the active agent in the oxidation, in the hemocyanin of the crayfish it is the copper. That such is really the case was readily shown by the introduction of a crystal of ferric chloride or copper sulphate into some of the blank control tests. Such "salted" controls reacted positively, before and after boiling.

The tests described proved two things: (1) The oxidation tests for hemoglobin do not serve to differentiate between this and other respiratory proteins, or between the blood of Vertebrates and Invertebrates. (2) They proved the presence of a respiratory protein in insects.

Two possibilities at once presented themselves,—that the carrier in question could be either hemoglobin, or hemocyanin; or both, as in some

mollusks. It was definitely ascertained, however, that in only a very few insects could hemoglobin be the respiratory protein. In most insects, if present at all, it was found only in infinitesimal quantities, and therefore negligible as a respiratory factor. This left the alternative of hemocyanin. This respiratory protein has been reported for a number of higher Crustacea and some Arachnida (Scorpion, Limulus). It is also widely known among the Mollusca, and there is no valid reason to assume that it might not be present in other groups of animals, including the insects.

To ascertain if this is the case, both direct and indirect methods were resorted to in this study. Unfortunately, no direct method for the recognition of hemocyanin is known such as the hemin test for hemoglobin. A large number of experiments were attempted to find such a reagent, but all were unsuccessful. Hence an indirect method was adopted.

In hemocyanin copper forms the nucleus of the respiratory compound. If the presence of copper could be shown in insect blood in amounts comparable to the copper content of equal quantities of crayfish blood,—then it would be logical to assume that this copper forms the nucleus of a respiratory protein similar to that of crayfish. Since, as already related, various tests indicated the presence of extremely minute quantities of hemoglobin, and since hemocyanin responded positively to the various hemoglobin tests, the latter were useless for differentiation between an iron and a copper compound. It therefore became necessary to separate the two, and to test separately for copper. This, of course, could be accomplished only after incineration of the tissues.

Among larger insects the blood and entire specimens, in small insects only whole specimens, were incinerated in the course of this study. The incinerations were begun in June 1920 and continued thru the summer and fall, as material became available. The usual analytical methods were followed: the ash was dissolved in hot dilute hydrochloric acid, a portion tested for iron, while the remainder was treated with excess of ammonia, precipitating the iron and dissolving the copper. The solution was then filtered, the filtrate concentrated by slow heating, acidulated with acetic acid, and tested for copper. Where the amount of ash was very small, the residue was redissolved and reprecipitated several times in order to obtain all the copper present.

As expected, iron showed heavily in all the incinerations, as it is universally present in animal cells. The thiocyanates were the chief reagents used in testing for this substance. Only qualitative tests were performed on copper, with notation of the approximate intensity of the reaction as compared with the control substance, namely crayfish blood. No exact quantitative estimates were possible, as the amounts dealt with

were microscopic. The reagents employed were Potassium ferrocyanide, Ammonium mercuric sulphocyanate, especially after the test drop had been inoculated with zinc salts (acetate or sulphate) or Caesium and Rubidium chloride; and finally, the Lead acetate—Potassium nitrite method for the formation of the triple nitrite Lead-Copper-Potassium. The ammonia so generally employed as a test for copper was not sufficiently delicate. It is sensitive only to about 1:2000, and the copper obtained in the few milligrams of ash was insufficient to react with it. The other reagents mentioned are sensitive to copper in dilutions up to 1:50000 and over, sufficiently so to give definite reactions.

The incinerations covered practically every order of insects (see Table II). The material incinerated was collected by the writer from Paradise Creek, two or three ponds, and the fields in the immediate vicinity of the university at Moscow, Idaho; except no. 45, Sialis larvae, which were obtained from Lake Mendota, Madison, Wis., and kindly sent me by Prof. Chancey Juday, of the Wisconsin Geological and Natural History Survey.

TABLE II. COPPER IN INSECTS

Name	Stage	Tissue	No. of Incinerations	Result Cu	Remarks
Coleoptera					
1. Dytiscus	larva	blood	2	pos	Equal to Cambarus
2. "	"	whole	2	pos.	slightly less
3. "	adult	blood	5	pos.	nearly equal
4. "	"	whole	4	pos.	slightly less
5. Gyrinus	adult	whole	1	pos.	less
6. Harpalus	adult	whole	1	pos.	less
7. Leptinotarsa	"	"	1	pos.	less than C.
Hymenoptera					
8. Apis mellifica	adult	whole	2	pos.	less than C.
9. Bombus sp.	"	"	1	pos.	less
10. Polistes	"	"	2	pos.	nearly equal
11. Formica	"	"	1	pos.	less
Lepidoptera					
12. Pieris rapae	larva	blood	1	pos.	about equal
13. " "	"	whole	1	pos.	about equal
14. " "	adult	whole	2	pos.	less
15. Noctuid moths	adult	whole	2	pos.	less
Diptera					
16. Musca domestica	larva	whole	2	pos.	slightly more
17. " "	adult	whole	2	pos.	about equal
18. Stomoxys	adults	"	2	pos.	less
19. Tachinid flies	"	"	1	pos.	less

TABLE II. COPPER IN INSECTS (*Continued*)

Name	Stage	Tissue	No. of Incinerations	Result Cu.	Remarks
Hemiptera					
20. Belostoma	yg. nymphs	blood	3	pos.	slightly less
21. "	yg. nymphs	whole	3	pos.	slightly less
22. "	adults	blood	6	pos.	about equal
23. "	"	whole	5	pos.	slightly less
24. Ranatra	"	"	1	pos.	" "
25. Gerris sp.	"	"	1	pos.	" "
26. Notonecta sp.	"	"	3	pos.	" "
27. Corixa sp.	"	"	2	pos.	" "
28. Aphis sp.	mixed	"	1	pos.	" "
Odonata					
29. Anax & Aeshna	yg. larvae	blood	2	pos.	less
30. Anax & Aeshna	yg. larvae	whole	2	pos.	less
31. Anax	larvae	blood	1	pos.	nearly equal
32. "	"	whole	1	pos.	less
33. Aeshna	"	blood	8	pos.	nearly equal
34. "	"	whole	7	pos.	" "
35. Sympetrum	"	blood	2	pos.	less
36. "	"	whole	2	pos.	"
37. Libellula	yg. larvae	whole	1	pos.	"
38. "	old larvae	whole	3	pos.	slightly less
39. Enallagma	yg. larvae	whole	1	pos.	less
40. "	late larva	"	3	pos.	about equal
41. "	adult	whole	2	pos.	less
Ephemeroidea					
42. Several spp.	nymphs	whole	1	pos.	slightly less
Trichoptera					
43. Several spp.	larvae	whole	1	pos.	nearly equal
Neuroptera					
44. Myrmeleon	adults	whole	3	pos.	equal or stronger
Megaloptera					
45. Sialis infumata	larvae	whole	1	pos.	nearly equal
Isoptera					
46. Termes sp.	mixed	whole	1	pos.	nearly equal
Orthoptera					
47. Gryllus	adult	whole	1	pos.	nearly equal
48. Ceuthophilus	"	"	1	pos.	less
49. Locusta	"	"	1	pos.	nearly equal
50. Melanoplus bivittatus	adult	"	2	pos.	nearly equal
51. Dissosteira	"	"	1	pos.	less

Total—34 species, 108 incinerations

A glance at the results in Table II shows positive reactions for copper in all insects incinerated, no matter what the stages chosen for ashing.

This universal presence of copper among the Insecta, not only in aquatic forms, but also in terrestrial species, indicates that it has an important function which hitherto has been overlooked. Its universal distribution is certainly not adventitious. Such a contingency might be explained for aquatic insects on the basis of the food supply (a large percentage of Crustacea), but would hardly apply to terrestrial insects, especially those among the latter which feed on plants only, or whose food is even more restricted, as in the case of honey bees. Furthermore, the copper present in the blood of many insects exists in practically the same proportions as in the blood of Crustacea. In Belostoma, for instance, five cc. of incinerated blood reacted to ammonia, showing a slightly fainter shade of blue than an equivalent amount of incinerated Cambarus blood. Indeed, copper was present to such extent, that an incineration of one cc. showed decisive reactions with all the reagents listed except ammonia. Other examples might be adduced, such as wasps and ant-lions. Here the ash of a single individual gave positive response to tests for copper.

Based on the foregoing results, the writer offers the hypothesis that the rôle of copper in insects is to form the nucleus of a blood protein,—namely a hemocyanin, similar in constitution to the known hemocyanins of Crustacea and mollusks; and that it serves a similar purpose, that of a respiratory pigment.

Based on experiments, recorded elsewhere, on the presence of oxygen and carbon dioxide in the blood of insects, the writer advances the further suggestion, that the function of this hemocyanin is parallel to that of hemoglobin,—namely, that the hemocyanin carries both oxygen and carbon dioxide, that compounds are formed in the respiratory cycle similar to the oxy- and carbohemoglobins. This second hypothesis has not been proved directly, but it is logical to assume that analogy of function in a respiratory protein, as hemocyanin is analogous to hemoglobin, should result in analogous compounds during the respiratory cycle. In short, it is reasonable to assume the formation of oxyhemocyanin with oxygen, and of carbohemocyanin with carbon dioxide.

From this standpoint, the various respiratory structures of advanced aquatic insects, such as the gill filaments and gill pouches of Trichoptera larvae and aquatic caterpillars, and the others referred to in the opening paragraph of this paper, acquire a real significance. If considered as of the category of true gills, to which type they undoubtedly belong, it is easy to understand how e ective they would be with a respiratory protein. Without such a pigment to fix the gases they would seem purposeless as structures, and inefficient physiologically.

III. Copper in Other Animals

As already stated, hemocyanin has been reported for mollusks, several species of higher Crustacea, scorpions and Limulus among Arachnida, and more recently for Coelenterates and fish. To ascertain whether or not it is found in the other classes of Arthropoda, the writer incinerated several species of plankton Crustacea, spiders, daddy-long-legs, centipeds, and millipeds (see Table III). In all of these copper was discovered in quantities equal to or exceeding the amount present in Cambarus blood.

As seen from this same table, examples of other phyla were also incinerated, including snails and slugs among mollusks, Lumbricus among Annulata, Ascaris among Nemathelminthes, Volvox among Protozoa, and human blood and snake blood for Chordata.

This material also was collected locally, except nos. 13-16 inclusive, which were collected from Wisconsin lakes, and kindly sent me by Prof. Chancey Juday, of the Wisconsin Geological and Natural History Survey.

The determination of copper in the blood of Cambarus has been noted repeatedly in this paper. The blood of this Crustacean was used constantly as a control for other incinerations. Only a small number of these controls are listed in the table. A second pigment has been reported for marine Decapoda, called Tetronerythrin, which is found also in our fresh water crayfish. The function of this pigment is unknown, altho it has been stated definitely that it is not a respiratory pigment. It is probable, however, that the pigment is used in the coloration and markings of the exoskeleton, and that it is carried passively in the bloodstream, similar to the pigments found in insect blood, and elaborated during the ecdysis. It is readily perceived in crayfish blood, from which it may be crystallized in orange-red crystals. Ordinarily it is not very abundant, but previous to moulting it is present in quantities sufficient to give a distinct pink or reddish color to the blood. Indeed, in larger crayfish, exceeding four inches in length, I have found the blood a bright red or scarlet, so that it resembled the diluted and aerated blood of a vertebrate. This blood clots in dark red masses, also resembling the clots of vertebrate blood. In "soft," freshly moulted crabs the blood appears transparent and contains little or none of this pigment.

Among species other than Arthropod Volvox furnished perhaps the greatest surprise by its show of copper, not merely as a trace, but in appreciable quantity: About 15 cc. of filtered Volvox were used in this incineration. That the reaction could not have been due to residual water is indicated by the fact that 100 cc. of water from the same pond showed not the slightest trace of this element. Its function in Volvox is problematical.

Ascaris furnished an additional surprise. Surely no one would suspect copper in an internal parasite. However, as barely a trace was found,

TABLE III. COPPER IN ANIMALS OTHER THAN INSECTS

Name	Stage	Tissue	No. of incinerations	Result	Remarks
Crustacea					
1. Cambarus sp.	1 in. long	blood	3	pos.	
2. "	1 in. long	whole	4	pos.	
3. "	2 in. long	blood	4	pos.	More Cu. than in No. 1.
4. "	2 in. long	whole	3	pos.	" " " " No. 3
5. "	3 in. long	blood	6	pos.	" " " " "
6. "	3 in. long	whole	3	pos.	" " " " No. 5
7. "	4 in. long	blood	5	pos.	" " " " "
8. "	4 in. long	whole	3	pos.	" " " " "
9. "	5 in. long	blood	3	pos.	" " " " No. 7
10. "	5 in. long	whole	2	pos.	" " " " "
11. Hyalella	adults	whole	2	pos.	less than No. 2
12. Cladocera & Copepods	adult	whole	1	pos.	" " "
13. Daphnia pulex	adults	whole	1	pos.	" " "
14. Microcystis	adults	whole	1	pos.	" " "
15. Copepods chiefly Limnocalanus)	adults	whole	1	pos.	" " "
16. Daphina pulex	adults	whole	1	pos.	" " "
Arachnida					
17. Argiope sp.	adult	whole	1	pos.	Equal to No. 9
18. Phalaena sp.	adults	whole	1	pos.	More than No. 9
19. Several spiders	adults	whole	1	pos.	Equal to No. 9
Myriapoda					
20. Millipeds spp.	adults	whole	1	pos.	More than No. 9
21. Centipeds spp.	adults	whole	1	pos.	More than No. 9
Annulata					
22. Lumbricus		whole	1	pos.	trace
Mollusca					
23. Physa sp.	mixed	whole	5	pos.	Equal to No. 3
24. Slugs	adult	whole	2	pos.	Equal to No. 4
Nemathelminthes					
25. Ascaris	♂ & ♀	whole	1	pos.	trace
Protozoa					
26. Volvox	mixed	whole	1	pos.	Equal to No. 2
Chordata					
27. Thamnophis sirtalis	adult	blood	1	pos.	trace in 2.8 gr.
28. Homo sapiens	adult	blood	1	neg.	about 1.6 gr. used.

and this due probably to mechanical storage, it would hardly be justifiable to attribute any physiological rôle to copper in this parasite. Its source is most probably the plant food taken in by the host.

In snails and slugs the copper undoubtedly occupies the same rôle that it has in squids, clams, and other mollusks. Lumbricus, like Ascaris, showed only a trace. Aside from leeches, this is the first time copper has been noted for an Annelid.

The fact that an abundance of copper was found in Myriapoda and in several representatives of the Arthropoda, in some even more than in the control substance, lends definite support to the assumption that for all Arthropoda copper is an essential element, and functions in the rôle of a respiratory protein in all members of this largest of phyla.

The discovery of copper in snake blood was due to pure chance. Two and eight tenths grams of snake blood besides a small quantity of human blood had been incinerated for another purpose. While waiting to utilize the ash at some later date it occurred to the writer to test for copper. (At the time I did not know of Rose and Bodansky's discovery of copper in marine fish.) The various tests were negative except two in which the test drop had been placed under alcohol vapor for several hours. No reaction showed in the first fifteen minutes, but after that indications of a positive reaction were noticeable. Later, when examined after an interval of several hours, the reactions showed definitely positive.

The ashed human blood referred to in the foregoing paragraph was also tested, but gave negative results. However, since the quantity was even less (1.83 gr.) than the snake blood used, the experiment is inconclusive.

IV. The Sources of Copper

For aquatic animals the source of copper is the slight amount in solution in the water. It is thus that mollusks and Crustacea obtain the copper necessary to their respiration. The soluble copper originates from the soil. Since the distribution of mollusks and Crustacea is universal, copper must likewise be available universally.

For terrestrial animals such as bees, wasps, caterpillars, moths, spiders, centipeds, etc., the soil cannot be considered as a direct source of copper. Their food consists largely of plants and animals, and perhaps minute droplets of water from wayside pools. It is evident that their copper must eventually come to them by way of their plant food. To determine this positively, a number of plants were incinerated and tested for copper (Table IV).

All plants reacted positively, but only to the more sensitive reagents, as the copper is present only in traces, not at all in amounts comparable to that of Arthropoda. All parts of the plant showed the presence of copper, with this difference: the fruit generally contained a less amount than the stem, leaves, or root. Because of the minimal amounts, its rôle in the plant is

probably not an active one, and its presence due to mechanical storage. As far as the relation of copper and plants is concerned, the copper ion is known to be highly toxic to plants, especially to the lower forms of plant life.

TABLE IV. COPPER IN PLANTS

Name	Part	No. of Incinerations	Result for Copper
1. Watermelon	Rind	2	Pos. trace
2. Pear	Leaves	2	pos. trace
3. Pear	fruit	2	pos. less than No. 2
4. Tomato	leaves and stem	2	pos. trace
5. Tomato	fruit	2	pos. less than No. 5
6. Potato	leaves	1	pos.
7. Lettuce	leaves	2	pos.
8. Red Beet	leaves	2	pos.
9. White Beet	leaves	2	pos.
10. Apple	leaves	2	pos.
11. Apple	fruit	2	pos. less than No. 11
12. Currant	leaves	2	pos.
13. Celery	stem	2	pos.
14. Clover	heads	1	pos.
15. Clover	leaves	2	pos. more than No. 14

Total—11 species, 30 incinerations.

Here in Moscow the copper content of the soil is very low. The surface soil is of aeolian origin and what copper it contains is brought by dust-storms from mountains 100 to 200 miles to the west in Washington and Oregon. In a quantitative estimate of copper in the soil 50 grams yielded only sufficient copper to permit a qualitative test.

Samples of water, each 3500 cc., taken from Paradise Creek during the summer and concentrated to 5 cc., likewise showed little more than traces of copper. A sample of water taken more recently (November) from under the ice showed a copper content of 0. 0187 gr. by the sulpho-cyanide method or approximately 0.00534 gr. per liter. The mud of Paradise Creek shows somewhat larger amounts, some of which may be due to organic matter.

V. DISCUSSION

In a recent paper on the occurrence of copper in marine organisms, Rose and Bodansky (1920) note the previous demonstration of copper in the following groups:

1. Echinodermata—starfish, urchins, sea-squirts.
2. Annulata—leech.
3. Crustacea—various Decapoda.
4. Arachnida—Scorpion, Limulus.

5. Mollusca—clams, oysters, snails, cuttle-fish, octopus.
6. Tunicata—Ciona.
7. Pisces—shark, 2 Teleosts.

To this list Rose and Bodansky add:

1. Coelenterata—jellyfish, Portuguese Man-of-War.
2. Mollusca—oysters, clams.
3. Curstacea—shrimps and crabs.
4. Pisces—Torpedo and sting ray, 12 Teleosts.

In all they add some 35 species, demonstrating for the first time that the copper in fish is not due to pathological causes. A survey of the groups studied in the present paper shows some interesting additions to the foregoing lists:

1. Protozoa—Volvox.
2. Nemathelminthes—Ascaris.
3. Mollusca—snails, slugs.
4. Annulata—Lumbricus.
5. Arthropoda.
    a. Crustacea—plankton, Cambarus, Hyalella.
    b. Arachnida—Phalaena, spiders.
    c. Myriapoda—centipeds, millipeds.
    d. Insecta—13 chief orders, over 35 species.
6. Chordata—snake.

To these must be added the occurrence of copper in higher plants.

Such a wide distribution of an element in a variety of living organisms, representing eight of twelve phyla, must have some significance. Its occurrence cannot be wholly adventitious, especially since it may be present in considerable quantities in the organism. Where present only in traces, it may well be ignored. In a number of forms its physiological rôle has been known for some time, altho physiologists believed that it was restricted to a few scattered species, and really was more or less an abnormality or rarity. Schulz, for instance (in Abderhalden 1910), states, "Hemocyanin occurs in the blood of higher Crustacea. It is present *only in a few members* (italics mine) of this class (Homarus, Maja, Portucuco, etc.)" Yet it is evident from the work of Rose and Bodansky and from the experiments herein noted that copper is not at all restricted to a few Decapoda among Crustacea, but that even the simplest and smallest Crustacea contain it.

Indeed, the writer, once he found positive indications of copper in a few species of insects, set out to test representatives of as many different groups as he could obtain locally. These were taken wholly at random, representing a variety of living conditions, from aquatic to parasitic, and entirely without regard to possible favorable results. (As a matter of fact,

in the end I purposely selected some of the least likely animals, such as Volvox, Ascaris, Lumbricus, and snakes.) This same attitude held for the work on lower Crustacea, spiders, and so on. The results, I believe, more than justified such a procedure, by indicating copper in the most unexpected places.

Yet this very fact of random selection is all the more convincing in a general application of the phenomena discovered. It signifies that these random selections are representative of whole groups and that what pertains to the few pertains also to the many. Since in these representative species copper has been found, and since in at least some of these few the physiological rôle of the copper has been definitely ascertained, it can be concluded that all or most remaining members of these groups also possess copper and that its physiological rôle is also similar. In other words, in at least the Mollusca and Arthropoda all species contain copper in appreciable quantities, and this copper functions as the nucleus of a respiratory protein, hemocyanin.

In thus extending particulate findings to entire groups of organisms I do not think I am overstepping the bounds of proper scientific conservatism. For copper was found in many groups, while in certain groups where a greater variety of material was available every species studied showed positive results. The uniformity of the results in these groups, unexpected as they were, is convincing and, I believe, warrants the above generalization.

VI. Summary

1. Both oxygen and carbon dioxide are present in insect blood in appreciable quantities. Insect blood aids in the transportation of gases, and the respiratory function is not confined to the tracheae.

2. Over 100 incinerations were made of insect blood, or whole specimens, and the ash tested for copper. Copper was found in all cases. The 35 species studied represent 13 of the chief orders of insects, in both larval and adult stages.

3. Copper is found in insect blood in quantities comparable to that of crayfish blood. Its rôle is therefore interpreted as being identical,—namely that it serves as the nucleus of a respiratory protein,—hemocyanin. Insects, therefore, have two sources of oxygen,—atmospheric air led directly to the tissues by way of the tracheae, and fixed oxygen carried by the respiratory protein of the blood.

4. Incinerations of plankton Crustacea, spiders, and centipeds gave positive results for copper, showing that copper is distributed among all classes of Arthropoda. It may therefore be regarded as an element essential to the physiological activity of Arthropods, its rôle being to act in a respiratory pigment for all members of this phylum.

5. As representatives of other phyla Volvox, Ascaris, snails and slugs, Lumbricus, human and snake blood were incinerated. All of these, except human blood, showed varying amounts of copper.

6. As sources of copper the water, soil, and plants were tested. All plant ash showed traces of copper. The water samples of this region showed only small quantities, while the soil showed varying amounts.

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